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Title

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Permalink

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Journal

Proceedings of the National Academy of Sciences of the United States of America,
115(31)

ISSN

0027-8424

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Publication Date

2018-07-01

DOI

10.1073/pnas.1803224115

Peer reviewed

Hippocampus-dependent emergence of spatial sequence coding in retrosplenial cortex

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Edited by Gyorgy Buzsáki, New York University Neuroscience Institute, New York, NY, and approved June 20, 2018 (received for review February 21, 2018)

Retrosplenial cortex (RSC) is involved in visuospatial integration and spatial learning, and RSC neurons exhibit discrete, place cell-like sequential activity that resembles the population code of space in hippocampus. To investigate the origins and population dynamics of this activity, we combined longitudinal cellular calcium imaging of dysgranular RSC neurons in mice with excitotoxic hippocampal lesions. We tracked the emergence and stability of RSC spatial activity over consecutive imaging sessions. Overall, spatial activity in RSC was experience-dependent, emerging gradually over time, but, as seen in the hippocampus, the spatial code changed dynamically across days. Bilateral but not unilateral hippocampal lesions impeded the development of spatial activity in RSC. Thus, the emergence of spatial activity in RSC, a major recipient of hippocampal information, depends critically on an intact hippocampus; the indirect connections between the dysgranular RSC and the hippocampus further indicate that hippocampus may exert such influences polysynaptically within neocortex.

retrosplenial cortex | hippocampus | spatial sequence coding | spatial learning | hippocampal indexing theory

The retrosplenial cortex (RSC) is a midline association region that integrates thalamic, (para)hippocampal, and neocortical information (1–5). Similar to the hippocampus (6), RSC is also essential for spatial learning and memory (7–9). Consistent with its proposed role in translating between world-centered and body-centered views (10), RSC neurons carry various navigation-related signals such as head direction, positional, and conjunctive allocentric and egocentric information (11–17). RSC neurons also show spatial activity resembling the activity of hippocampal CA1 place cells (15). The sources of spatial signals in RSC are unknown; however, hippocampus is an obvious possibility, and hippocampal lesions or inactivation impair immediate early gene expression in RSC (18, 19). On the other hand, both rodent and human studies have suggested the opposite direction of information flow, that RSC may send sensory and contextual information to the hippocampus (20, 21), possibly through RSC projections to the medial entorhinal cortex (22). Here we studied the emergence of spatial activity in RSC upon repeated exploration of the same environment and tested the impact of the hippocampus on this activity.

Results

We investigated RSC neuronal activity in mice in a head-fixed, treadmill assay (15, 23) (Fig. 1A). Fifteen adult transgenic mice specifically expressing calcium indicator GCaMP6 in excitatory neurons (24, 25) were divided into three experimental groups: control, unilateral hippocampal lesion, and bilateral hippocampal lesions ($n = 5$ in each group; NMDA lesion) (Fig. 1B). Mice in the lesion groups sustained extensive neuron/tissue loss in the dorsal hippocampal formation (Fig. 1C and *SI Appendix*, Fig. S1). Movement trajectories were similar across groups (average speed between 30- and 120-cm position: control, 15.4 ± 1.7 ; unilesion, 17.7 ± 1.8 ; bilesion, 18.9 ± 3.5 cm/s; all mean \pm SEM, $n = 5$ in each group; $P = 0.61$, one-way ANOVA) (Fig. 1D). We measured cellular activity in the superficial layers (100 μ m to 200 μ m deep)

of dysgranular RSC using two-photon calcium imaging (26) (*SI Appendix*, Fig. S2). We inferred activity from raw calcium fluorescence signals using deconvolution (*SI Appendix*, Fig. S2) (27). We studied the degree to which RSC neurons encode the animal's position on the treadmill. Consistent with our previous work (15), a substantial fraction of RSC neurons showed repeated activation at specific positions on the treadmill (Fig. 1E and *SI Appendix*, Fig. S1D), similar to the activity of hippocampal place cells.

Hippocampal projections to RSC are mostly ipsilateral (2); we therefore tested whether a unilateral hippocampal lesion disrupts “place” cell activity in ipsilateral RSC. We compared activity in the lesioned and intact hemispheres measured from the same animals. Unilateral hippocampal lesion had no discernible impact on RSC spatial activity (Fig. 2). RSC neuronal ensembles showed sequential activation that was locked to position during movement in both the intact and lesioned hemispheres (Fig. 2A and B). To quantify the encoding of spatial information by RSC neuronal population, we built a Bayesian decoding model (*SI Appendix*, *Methods* and Fig. S3A) to predict the animal's position from all imaged neurons using separate sets of trials for training and testing (Fig. 2A and B) (28). We observed no significant difference in the place cell fraction ($P = 0.30$, paired t test) or the position decoding error ($P = 0.98$, paired t test) between the lesioned and intact hemispheres (Fig. 2C).

Significance

Retrosplenial cortex (RSC) is a major relay of hippocampal formation output to other neocortical areas and is critical for spatial and some other forms of learning. We show here that the sparse, orthogonal, “place cell” sequence activity in RSC develops gradually over several days and is severely attenuated by hippocampal damage. These data support the theory that hippocampus endows RSC (and possibly other cortical areas) with an index-like, continuous representation of the context in which events occur, that could support coordinated retrieval of recent memory.

Author contributions: D.M., V.B., M.H.M., and B.L.M. designed research; D.M., A.R.N., and J.S. performed research; D.M. analyzed data; and D.M., V.B., and B.L.M. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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Data deposition: The data related to this work has been deposited on Gin and is available at https://web.gin.g-node.org/dunmao/RSC_HPC.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1803224115/-DCSupplemental.

Published online July 16, 2018.

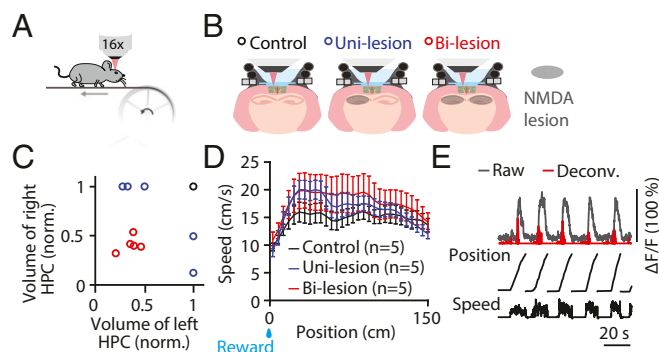


Fig. 1. Experimental design and retrosplenial place cell activity. (A) Treadmill locomotion assay. Two-photon calcium imaging was performed in head-fixed mice running on a treadmill belt endowed with tactile cues. (B) Cellular imaging of neuronal activity through a glass window in RSC in both hemispheres in mice with intact hippocampus (control), unilateral hippocampal lesion (unilesion), and bilateral hippocampal lesions (bilesion). (C) Scatter plot of the remaining volume (normalized) of the hippocampus in the left and right hemispheres. Colors correspond to animal groups shown in B. (D) Mean movement speed profiles as a function of position for the three experimental groups. Error bars are SEM over animals. (E) Calcium time courses (raw and deconvolved) of an example RSC place cell. Position and speed traces are shown below. deconv, deconvolved; norm, normalized.

While unilateral hippocampal lesions did not disrupt ipsilateral RSC spatial activity, RSC could also receive spatial information from the intact, contralateral hemisphere (2). We compared RSC place cell activity between intact animals and animals with bilateral hippocampal lesions. Indeed, bilateral hippocampal lesions significantly impaired place cell activity in RSC (Fig. 3). RSC spatial sequence activity was severely disrupted by bilateral hippocampal lesions (Fig. 3A and B). The fraction of RSC neurons showing stable place fields dropped dramatically in animals with bilateral lesions (place cell fraction: control, 0.50 ± 0.02 ; bilesion, 0.29 ± 0.04 ; mean \pm SEM, $n = 5$ each group; $P = 0.0005$, one-tailed t test) (Fig. 3C). An alternative, spatial information-based criterion of place cell selection yielded similar results (SI Appendix, Fig. S3 B–G). Position estimates inferred from Bayesian decoding of population activity (using all cells) were also severely disrupted in bilaterally lesioned animals (decoding error: control, 14.60 ± 1.98 cm; bilesion, 27.68 ± 2.20 cm; mean \pm SEM; $P = 0.0011$, one-tailed t test) (Fig. 3D). These effects were not explained by other variables such as neuron or trial counts (SI Appendix, Fig. S3 H and I). Notably, while the proportion of place cells was reduced in animals with bilateral hippocampal lesions, the properties of neurons with place cell activity were similar (place field width: $P = 0.28$; spatial information: $P = 0.21$, two-tailed t test) (Fig. 3 E and F and SI Appendix, Figs. S3J and S4). The extent of hippocampal lesions may explain the residual place cell activity observed in bilaterally lesioned animals, since the magnitude of the effect was proportional to the proportion of hippocampal damage (SI Appendix, Fig. S3J). These results indicate that the hippocampus is necessary for the expression of place cell activity in RSC.

We next investigated how RSC place cell activity emerges over repeated exploration of the same environment and what the role of the hippocampus is in this process. We monitored the activity of the same RSC neuronal population over a period of 2 wk to 3 wk in daily imaging sessions (control, $n = 4$; bilesion, $n = 4$). Activity from the same neuronal cell bodies could be imaged across days (Fig. 4A and SI Appendix, Fig. S5 A and B). Place cell activity increased gradually with experience in the control group but not in mice with bilateral hippocampal lesions (place cell fraction change: control, $r = 0.96$, $P = 0.0006$; bilesion, $r = 0.61$, $P = 0.17$) (Fig. 4D).

Similar to what is observed in the hippocampus (29), RSC population representations of position on the track changed dynamically across days (Fig. 4B), and more adjacent days had more highly correlated population activity as experience increased (Fig. 4 B and C). The control group showed more repeatable population representations across days than the bilateral lesion group (population vector correlations: control, 0.19 ± 0.02 ; bilesion, 0.09 ± 0.02 , $P = 0.02$, two-tailed t test) (Fig. 4E and SI Appendix, Fig. S5C). Position tuning curves (occupancy-normalized neuronal responses as a function of position) of individual neurons were also more correlated between days in control mice than in bilateral lesion mice ($P = 0.02$, two-tailed t test) (Fig. 4E). These effects were not explained by the number of place cells included in the calculation (SI Appendix, Fig. S5F). We did not observe a difference in spatial activity correlations (population vector correlations or position tuning correlations of individual neurons) between the two hemispheres in mice with unilateral hippocampal lesion (SI Appendix, Fig. S5 D and E). These results indicate that RSC population representation of spatial context improves progressively over time and that the hippocampus is necessary for the emergence of spatial sequence coding in RSC.

Discussion

Our data indicate that the spatial context coding in RSC improves with experience, and this process relies on instructive signals from the hippocampus. This may reflect a direct impact

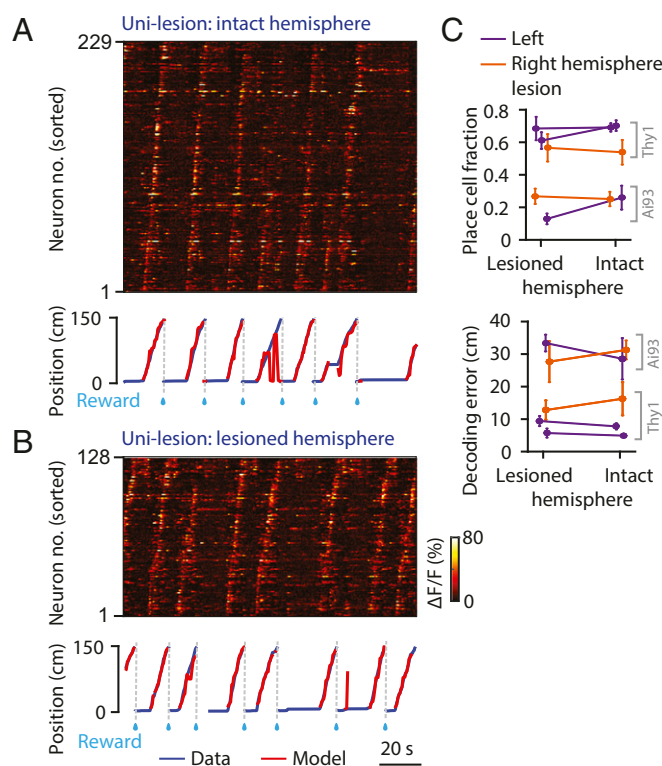


Fig. 2. Unilateral hippocampal lesion does not impair ipsilateral RSC place cell activity. (A) (Top) Raw calcium time courses of 229 simultaneously imaged RSC neurons in the intact hemisphere of an example unilesion mouse. Neurons were sorted by the positions that elicited their maximum responses. (Bottom) Real (blue) and Bayesian decoded (red) position traces. (B) The same as A but for the lesioned hemisphere RSC of the same mouse. (C) Mean place cell fractions and position decoding errors in unilesion mice. Note that two mouse lines were used here (Thy1 and Ai93). Error bars are SEM over sessions. For unknown reasons, there were intrinsic differences between these lines in the observed place cell fractions.

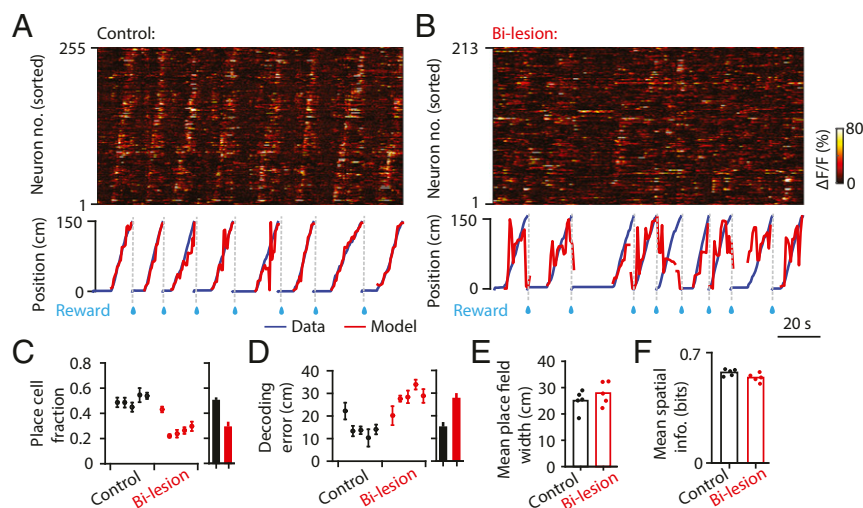


Fig. 3. Bilateral hippocampal lesions severely impair place cell activity in RSC. (A) (*Top*) Raw calcium time courses of 255 simultaneously imaged RSC neurons from an example control mouse. Neurons were sorted by the positions that elicited their maximum responses. (*Bottom*) Real (blue) and Bayesian decoded (red) position traces. (B) The same as A but from an example bilateral lesion mouse. (C) (*Left*) Mean place cell fractions of individual mice in the control and bilesion groups (all Thy1 mice). Error bars are SEM over sessions. (*Right*) Bar plots of the average place cell fractions for the two groups. Error bars are SEM over animals. (D) The same as C but for decoding errors. (E) Bar plot of the mean place field width. Colored dots correspond to the mean place field width of individual mice in the control and bilesion groups. (F) The same as E but for the mean spatial information of identified place cells in each animal.

of the hippocampus or an indirect effect through intermediate regions after lesioning the hippocampus. Indeed, the specific RSC subregion we studied, the dysgranular RSC, receives weak direct hippocampal input (1, 3, 4). Our results demonstrate the importance of the hippocampus in shaping neocortical activity. The pronounced experience-dependent spatial activity observed may reflect a general principle of the influences of hippocampal outflow on the association neocortex in terms of spatiotemporal contextual processing. Sequential activation of large groups of

neurons has been observed in several cortical regions, including the posterior parietal and prefrontal cortices (30, 31). These sequences reflect information processing along the spatial and/or temporal dimensions, with concurrent sensory experience and events superimposed. Being uniquely situated at the intermediate layer within the default mode network (DMN) (32), RSC may be critical for episodic memory processes by mediating functional interactions between the cortical and subcortical DMN subsystems (33, 34). The RSC may play a critical role in

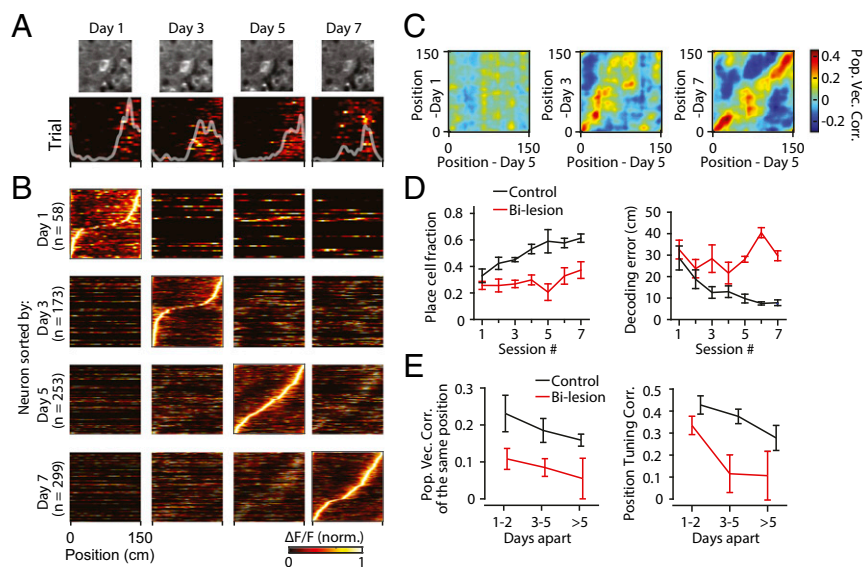


Fig. 4. Hippocampal destruction disrupts experience-dependent emergence of spatial coding in RSC. (A) Average fluorescence images (centered on the target neuron) and position activity maps of the target neuron imaged on days 1, 3, 5, and 7. Position tuning curves (white traces) are overlaid on the position activity maps. (B) Sorted, trial-averaged position activity maps for all RSC place cells from an example control mouse on days 1, 3, 5, and 7. Same neuronal population was imaged across days. Neurons were selected and sorted by corresponding days. (C) Population vector correlation matrices between days for data shown in B. (D) Mean place cell fractions and decoding errors as a function of imaging session for the control and bilateral lesion animals. Error bars are SEM over animals. (E) Mean population vector correlations of the same position (distance < 15 cm, dashed area in [S1 Appendix, Fig. S5C](#)) and mean position tuning (white traces in A) correlations for all place cells as a function of different imaging intervals. Error bars are SEM over animals. norm, normalized; Pop Vec Corr, population vector correlation.

the transfer of hippocampal place/memory sequence codes to other regions of the neocortex, to associate information across different cortical modalities (35–37) and to guide complex behaviors (38).

Methods

All animal procedures were performed in compliance with protocols approved by the ethical research committee of the University of Lethbridge. Fifteen adult male and female transgenic GCaMP6 mice [20 g to 25 g, 2 mo to 4 mo old at the time of surgery, including 13 Thy1 GCaMP6s GP4.3 mice and 2 Ai93 (TITL-GCaMP6f) || CaMK2a-tTA || Rasgrf2-2A-dCre mice] were used in this study. Mice were divided into three experimental groups: sham lesion (control, $n = 5$ Thy1 mice), unilateral hippocampal lesion ($n = 3$ Thy1 mice and $n = 2$ Ai93 mice), and bilateral hippocampal lesions ($n = 5$

Thy1 mice). Mice were habituated and trained to run on a linear treadmill track with head fixed.

Full methods can be found in *SI Appendix, Methods*.

ACKNOWLEDGMENTS. We thank V. Lapointe and A. Demchuk for help with histology, and F. Battaglia for comments on the manuscript. This research was supported through the Alberta Innovates-Health Solutions Polaris award (to B.L.M.) and a graduate studentship (to D.M.), Natural Sciences and Engineering Research Council of Canada Discovery Grant 40352 (to M.H.M.) and RGPIN-2017-03857 (to B.L.M.), Research Foundation-Flanders (Fonds voor Wetenschappelijk Onderzoek - Vlaanderen) Grant G0D0516N (to V.B.), KU Leuven Research Council Grant C14/16/048 (to V.B.), National Science Foundation Grant 1631465 (to B.L.M.), Canada Foundation for Innovation Grant 33598 (to M.H.M. and B.L.M.), and Defense Advanced Research Projects Agency Grant HR0011-18-2-0021 (to B.L.M.).

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